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(54) Title: PROCESS FOR ISOLATING NUCLEIC ACID WITH CHAOTROPE AGENTS AND AMMONIUM COMPOUNDS

(57) Abstract: A process for isolating nucleic acid from a nucleic acid-containing sample, which comprises: (a) providing a chaotrope; (b) providing a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope; (c) providing a source of NH₄⁺ or NH₃; (d) contacting the sample with the nucleic acid binding solid phase in the presence of a liquid phase comprising the chaotrope and the NH₄⁺ or NH₃; and (e) optionally separating the solid phase with the nucleic acid bound thereto from the liquid phase.

PROCESS FOR ISOLATING NUCLEIC ACID WITH CHAOTROPE AGENTS AND AMMONIUM COMPOUNDS

Field of the Invention

The present invention relates to a process for isolating nucleic acid from a nucleic acid-containing sample, and to a kit therefor.

Background to the Invention

Procedures involving nucleic acids such as DNA and RNA continue to play a crucial role in biotechnology. Nucleic acid detection and manipulation including hybridisation, amplification, sequencing and other processes generally require nucleic acid to have been isolated from contaminating material. Where a nucleic acid-containing sample is a biological sample, contaminating material may include proteins, carbohydrates, lipids and polyphenols. Accordingly, a variety of approaches have hitherto been used in the isolation of DNA or RNA.

Early methods of isolating nucleic acid involved a series of extractions with organic solvents, involving ethanol precipitation and dialysis of the nucleic acids. These early methods are relatively laborious and time-consuming and may result in low yield. Isopropanol may also be used in the precipitation of the nucleic acid.

US5234809 describes a procedure to isolate DNA from biological samples which uses a chaotropic agent together with a silica based nucleic acid binding solid phase. Guanidine hydrochloride at pH 3 to 5 or guanidine thiocyanate at higher pH, combined with other salts, is

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used as the chaotropic agent. After binding of the DNA to the solid surface, the solid phase may be washed with the chaotropic agent to remove any biological contamination followed by treatment with 70% ethanol to remove the chaotrope. The DNA is eluted using water.

A variant on this methodology is described in US6027945. Here, a method is described which also uses a silica-based nucleic acid binding solid phase in the presence of a chaotrope to isolate nucleic acid. According to this method, the silica-based solid phase is magnetic, thereby facilitating separation of the solid phase containing the target nucleic acid from the liquid phase containing contaminants upon application of a magnetic field.

WO96/18731 also uses magnetic particles to bind nucleic acid. In this disclosure the magnetic particles are polystyrene-based and polyurethane-coated and a detergent is used instead of a chaotrope.

In spite of the advances made using nucleic acid binding solid phases, the yield of target material can sometimes be undesirably low. The present invention addresses this disadvantage of the prior art.

Summary of the Invention

Accordingly, in a first aspect, the present invention provides a process for isolating nucleic acid from a nucleic acid-containing sample, which comprises:

- (a) providing a chaotrope;

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- (b) providing a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope;
- (c) providing a source of NH_4^+ or NH_3 ;
- (d) contacting the sample with the nucleic acid binding solid phase in the presence of a liquid phase comprising the chaotrope and the NH_4^+ or NH_3 ; and
- (e) optionally separating the solid phase with the nucleic acid bound thereto from the liquid phase.

In a second aspect, the present invention provides a kit for isolating nucleic acid from a nucleic acid-containing sample, which kit comprises:

- (a) a chaotrope;
- (b) a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope; and
- (c) a source of NH_4^+ or NH_3 .

It has surprisingly been found that the presence of NH_4^+ or NH_3 in the process for isolating nucleic acid gives an increased yield of nucleic acid compared to cases where NH_4^+ or NH_3 are absent.

Without wishing to be bound by theory, it is thought that the addition of ammonia or ammonium to, say, the chaotropic binding solution, causes the pH to increase by one unit (i.e. from 7.5 to 8.5). However, the resulting increased yield of isolated nucleic acid is not believed to be purely a pH effect. If the pH of the chaotropic solution is increased to 8.5 simply by the addition of alkali, this does not affect the yield of isolated nucleic acid. However, the pH of the solution in the presence of ammonia

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or ammonium does have an effect on the increased yield of the isolated nucleic acid. Adjusting, say, the chaotropic solution containing ammonia or ammonium back to pH 7.5 with acid does tend to reduce the yield of isolated nucleic acid. Moreover, if the pH exceeds 9.5, the yield of isolated nucleic acid tends to drop. Accordingly, it is preferred that the step of contacting the sample with the nucleic acid binding solid phase in the presence of the NH_4^+ or NH_3 is conducted at a pH in the range 8.5 to 9.5.

Instead of NH_4^+ or NH_3 , an amine may be used, preferably a primary amine.

The nucleic acid-containing sample typically comprises a biological sample such as a cellular sample. The biological sample may or may not need to be pretreated, depending on its structure. For example, in the case of plant or fungal cells or solid animal tissue, pretreatment would be required as is known in the art. Samples stored in the form of a solid phase such as a paraffin section may also need pretreatment. Samples may be from foodstuffs, environmental samples or clinical samples and may contain prokaryotic or eukaryotic cells or other moieties such as mycoplasmas, protoplasts or viruses. Blood products are an important area for nucleic acid isolation and the present invention is particularly applicable to whole blood and other blood products such as plasma, serum and buffycoat.

The nucleic acid to be isolated may be DNA, RNA or a modified form thereof. Where the nucleic acid is DNA, this

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may be ds or ss DNA. Where the nucleic acid is RNA, this may be rRNA, mRNA or total RNA.

The chaotrope generally comprises a chaotropic ion provided at a concentration sufficiently high to cause the nucleic acid to lose its secondary structure and, in the case of double-stranded nucleic acids, to melt. Chaotropes are thought to disrupt hydrogen-bonding in water so as to make denatured nucleic acid more stable than its undenatured counterpart. The chaotrope typically comprises a guanidinium salt, urea, or an iodide, chlorate, perchlorate or (iso)thiocyanate. Preferred chaotropes include guanidinium thiocyanate, and guanidinium hydrochloride.

The concentration of chaotrope typically present when contacted with the sample is in the range 2M to 8M.

The nucleic acid binding solid phase must be capable of binding nucleic acid in the presence of the chaotrope but is not limited to any specific material. Various materials are now known as nucleic acid binding solid phases and these include silica-based materials such as those described in US5234809, polymeric materials including latex and polystyrene-based materials such as those described in WO96/18731 and other materials such as glasses.

The form of the solid phase includes sheets, sieves, sinters, webs and fibres. Particles are particularly useful as these may be packed in a column or used in suspension and have high binding capacity. Magnetic particles are particularly preferred because of the ease

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with which they merely separated from an associated liquid phase in a magnetic field. Typical materials for use in magnetic particles include magnetic metal oxides especially the iron oxides. Useful magnetic oxides include iron oxides in which, optionally all or a part of the ferrous iron thereof is substituted with a divalent transition metal such as cadmium, chromium, cobalt, copper, magnesium, manganese, nickel, vanadium and/or zinc. Silica-based magnetic particles useful in the present invention include those described in US6027945 and US5945525.

The source of NH_4^+ or NH_3 is typically an ammonia solution although other possible sources include those capable of generating ammonia by a chemical reaction or transformation. In order for the NH_4^+ or NH_3 to be present when the sample is contacted with the nucleic acid binding solid phase, there is no particular limitation on how the NH_4^+ or NH_3 should be provided. Conveniently, the NH_4^+ or NH_3 can be provided with the chaotrope, although the technical effect provided by the invention also allows the NH_4^+ or NH_3 to be provided with the solid phase or even the sample. A potential advantage does arise if the chaotrope and NH_4^+ or NH_3 are provided together, however. The process according to the invention may further comprise a lysis step comprising subjecting the biological sample to conditions to lyse the sample. This is typically carried out so as to disrupt cells and release their nucleic acid. Lysis conditions conveniently involve the presence of a detergent. It is thought potentially advantageous for the NH_4^+ or NH_3 to be present during the lysis step as this may have the beneficial effect of increasing yield of nucleic

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acid during this step. It is also convenient to have the chaotrope present at the same time as this can help the lysis step. Accordingly, where the chaotrope and the NH_4^+ or NH_3 are provided together as a solution, this solution can be used to treat the biological sample during the lysis step.

The step of separating the solid phase with the nucleic acid bound thereto from the liquid phase is generally required in order to remove contaminants in the liquid phase. Further washing steps may be applied to the solid phase at this point. Any conventional separation step for separating solid phase from liquid phase is applicable, including centrifugation and decanting of the liquid phase from the pelleted solid phase or using a column in which the solid phase is packed and the liquid phase passed through. Where the magnetic solid phase is used, this facilitates separation, which can be carried out in the presence of a magnetic field.

Depending on the form in which the isolated nucleic acid is required, a further elution step can be provided. In some cases it may be satisfactory for the nucleic acid to remain bound to the solid phase. This may be the case if further manipulations of the nucleic acid on a solid phase are required, such as an amplification step. Equally, the nucleic acid may be eluted from the solid phase by applying an elution solution, which may simply be water or a buffer.

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Brief Description of the Drawings

The present invention is now described in more detail, by way of example only, with reference to the following Example and accompanying figure.

Figure 1 shows a graph of DNA yield plotted against amount of ammonia in a chaotropic lysis and binding solution.

Detailed Description**Example**

The magnetic particles. Magnetic Silica particles were obtained in accordance with UK patent application no. 0116359.1 filed on 4th July 2001.

The chaotropic lysis and binding solution. To 130 g Guanidine thiocyanate (Sigma) was added 95 ml 0.1 M TRIS HCl pH 7 (Sigma) + 8 ml 0.5 M EDTA (Invitrogen) and 2.5 g tween-20 (Sigma). The solution was heated on a water bath at 30°C for 1 h. The pH of the solution was 7.5. This solution was used as the reference sample to which no ammonia or ammonium was added. To this solution was added 16 ul 5% NH₃ (Merck)/ml chaotropic solution to leave pH at 8.5 as the ammonia or ammonium chaotropic solution described.

The chaotropic wash I solution. To 120g Guanidine hydrochloride (Sigma) was added water to a total of 160 ml (7.5M).

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The ethanol based wash II solution. To 10 ml 4M NaCl (Sigma) was added 100 ul 96% EtOH. To 800µl of this solution was added 100 ul water.

The DNA binding procedure. 50, 100 and 150 ul of whole blood (WBC 7.7) were added to 720 ul of the chaotropic lysis and binding solution. After 1 min, magnetic silica beads were added (ca 15 mg) and the solution was allowed to incubate for 10 min whereafter the magnetic beads were collected on a magnet. The beads were resuspended in washing solution I and again collected on a magnet. This step was repeated once. The beads were resuspended and washed in washing solution II and collected on a magnet. This step was repeated once. Finally, 100 ul water was added to the beads and they were resuspended at ambient temperature for ca 2 min. The beads were collected on a magnet and the supernatant was transferred to a new tube. The yield of isolated DNA was measured on a Spectrophotometer (Perkin Elmer, Lambda EZ 201).

The results are shown in Figure 1, in which DNA yield (y-axis) is plotted in arbitrary units against µl of 5% ammonia in the chaotropic lysis and binding solution. The lysis volume is fixed at 760µl and the solid phase is fixed at 15mg.

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CLAIMS:

1. A process for isolating nucleic acid from a nucleic acid-containing sample, which comprises:
 - (a) providing a chaotrope;
 - (b) providing a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope;
 - (c) providing a source of NH_4^+ or NH_3 ;
 - (d) contacting the sample with the nucleic acid binding solid phase in the presence of a liquid phase comprising the chaotrope and the NH_4^+ or NH_3 ; and
 - (e) optionally separating the solid phase with the nucleic acid bound thereto from the liquid phase.
2. A process according to claim 1, which further comprises a step of eluting the nucleic acid from the solid phase.
3. A process according to claim 1 or claim 2, wherein the sample comprises a biological sample.
4. A process according to claim 3, wherein the biological sample comprises a cellular sample.
5. A process according to claim 3 or claim 4, which further comprises a lysis step comprising subjecting the biological sample to conditions to lyse the sample.
6. A process according to claim 5, wherein the NH_4^+ or NH_3 is present during the lysis step.

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7. A process according to any preceding claim, wherein the nucleic acid comprises DNA.
8. A process according to claim 7, wherein the DNA comprises ds or ss DNA.
9. A process according to any of claims 1 to 6, wherein the nucleic acid comprises RNA.
10. A process according to claim 9, wherein the RNA comprises rRNA, mRNA or total RNA.
11. A process according to any preceding claim, wherein the chaotrope comprises a guanidinium salt, urea, or an iodide, chlorate, perchlorate or (iso)thiocyanate.
12. A process according to any preceding claim, wherein the nucleic acid binding solid phase comprises a silica-based solid phase.
13. A process according to any preceding claim, wherein the solid phase is magnetic.
14. A process according to any preceding claim, wherein the source of NH_4^+ or NH_3 comprises a solution of ammonia.
15. A process according to any preceding claim, wherein the source of NH_4^+ or NH_3 and the chaotrope are provided together as a solution.

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16. A kit for isolating nucleic acid from a nucleic acid-containing sample, which kit comprises:

- (a) a chaotrope;
- (b) a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope; and
- (c) a source of NH_4^+ or NH_3 .

17. A kit according to claim 16, which further comprises a solution for eluting the nucleic acid from the solid phase.

18. A kit according to claim 16 or claim 17, which further comprises a lysis solution for lysing biological samples.

19. A kit according to any of claims 16 to 18, wherein the nucleic acid binding solid phase comprises a silica-based solid phase.

20. A kit according to any of claims 16 to 19, wherein the solid phase is magnetic.

21. A kit according to any of claims 16 to 20, wherein the source of NH_4^+ or NH_3 comprises a solution of ammonia.

22. A kit according to any of claims 16 to 21, wherein the source of NH_4^+ or NH_3 and the chaotrope are provided together as a solution.

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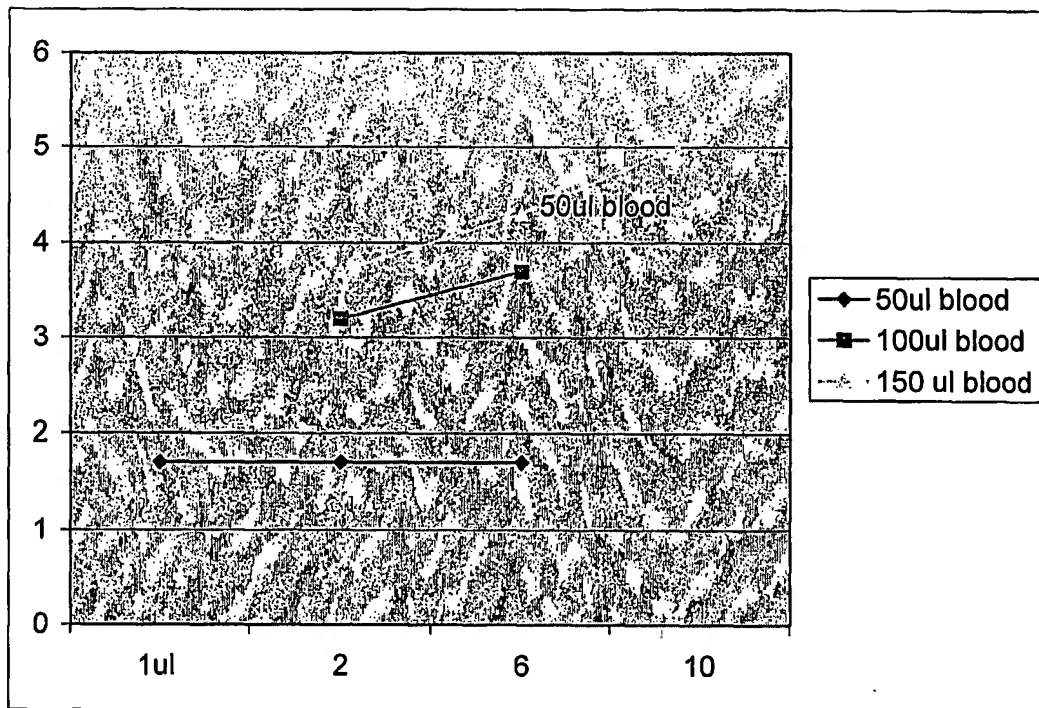


Figure 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/01202

| A. CLASSIFICATION OF SUBJECT MATTER | | |
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| IPC 7 | C07H21/00 | G01N33/553 C01B21/68 C12N1/08 C12N15/10 C12Q1/70 C12Q1/68 |
| According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED | | |
| Minimum documentation searched (classification system followed by classification symbols) | | |
| IPC 7 C07H G01N C12N C12Q | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | |
| Electronic data base consulted during the International search (name of data base and, where practical, search terms used) | | |
| EP0-Internal | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | STANLEY B. PRUSINER ET AL: "Thiocyanate and hydroxyl ions inactivate the scrapie agent" PROC. NATL. ACAD. SCI., vol. 78, no. 7, July 1981 (1981-07), pages 4606-4610, XP002245639 tables 1-3 | 1-22 |
| X | EP 0 969 090 A (QIAGEN GMBH) 5 January 2000 (2000-01-05) claim 8 | 1-22 |
| Y | US 6 270 970 B1 (SMITH CRAIG E ET AL) 7 August 2001 (2001-08-07) claim 11 | 1-22 |
| -/-- | | |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. | | |
| * Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family | | |
| Date of the actual completion of the international search | | Date of mailing of the international search report |
| 26 June 2003 | | 29. 07. 2003 |
| Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | | Authorized officer FERNANDO FARIETA /EÖ |

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/01202

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| Y | US 6 090 288 A (BERGLUND ROLF ET AL) 18 July 2000 (2000-07-18) claim 1 | 1-22 |
| Y | EP 0 540 170 A (BECTON DICKINSON CO) 5 May 1993 (1993-05-05) formula I | 1-22 |
| Y | EP 1 018 549 A (HAYASHIZAKI YOSHIHIDE ;RIKAGAKU KENKYUSHO (JP)) 12 July 2000 (2000-07-12) claim 4 | 1-22 |
| A | HONG-KHANH NGUYEN ET AL: "Smoothing of the thermal stability of DNA duplexes by using modified nucleosides and chaotropic agents" NUCLEIC ACIDS RESEARCH, vol. 27, no. 6, 1999, pages 1492-1498, XP002245640 Discussion | 1-22 |
| A | CHRISTOPHER E. DAHLE ET AL: "Isolation of RNA from Cells in Culture Using Catrimox-14TM Cationic Surfactant" BIOTECHNIQUES, vol. 15, no. 6, 1993, pages 1102-1105, XP002245641 tables 1-3 | 1-22 |
| A | US 5 234 809 A (ADRIAANSE HENRIETTE M A ET AL) 10 August 1993 (1993-08-10) claims 1-14 | 1-22 |
| A | US 6 027 945 A (SMITH CRAIG E ET AL) 22 February 2000 (2000-02-22) claim 1 | 1-22 |
| A | WO 87 06621 A (GILLESPIE DAVID) 5 November 1987 (1987-11-05) claims 1-43 | 1-22 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 03/01202

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|-------------------------------------------|----|---------------------|----------------------------|---------------------|
| EP 0969090 | A | 05-01-2000 | EP 0969090 A1 | 05-01-2000 |
| | | | WO 9961603 A1 | 02-12-1999 |
| | | | EP 1088064 A1 | 04-04-2001 |
| | | | JP 2002516094 T | 04-06-2002 |
| | | | US 2003032147 A1 | 13-02-2003 |
| ----- | | | | |
| US 6270970 | B1 | 07-08-2001 | AU 4841500 A | 05-12-2000 |
| | | | CA 2372521 A1 | 23-11-2000 |
| | | | EP 1179056 A1 | 13-02-2002 |
| | | | JP 2002543980 T | 24-12-2002 |
| | | | WO 0070041 A1 | 23-11-2000 |
| | | | US 2002001812 A1 | 03-01-2002 |
| ----- | | | | |
| US 6090288 | A | 18-07-2000 | AU 713635 B2 | 09-12-1999 |
| | | | AU 1818097 A | 02-09-1997 |
| | | | CA 2242927 A1 | 21-08-1997 |
| | | | EP 0888157 A1 | 07-01-1999 |
| | | | JP 2000504625 T | 18-04-2000 |
| | | | WO 9729825 A1 | 21-08-1997 |
| ----- | | | | |
| EP 0540170 | A | 05-05-1993 | US 5329000 A | 12-07-1994 |
| | | | CA 2079749 A1 | 01-05-1993 |
| | | | DE 69206580 D1 | 18-01-1996 |
| | | | DE 69206580 T2 | 05-06-1996 |
| | | | EP 0540170 A1 | 05-05-1993 |
| | | | US 5534054 A | 09-07-1996 |
| ----- | | | | |
| EP 1018549 | A | 12-07-2000 | JP 11092494 A | 06-04-1999 |
| | | | EP 1018549 A1 | 12-07-2000 |
| | | | US 6342387 B1 | 29-01-2002 |
| | | | WO 9915645 A1 | 01-04-1999 |
| | | | US 2002025572 A1 | 28-02-2002 |
| ----- | | | | |
| US 5234809 | A | 10-08-1993 | NL 8900725 A | 16-10-1990 |
| | | | AT 156830 T | 15-08-1997 |
| | | | AU 641641 B2 | 30-09-1993 |
| | | | AU 5215390 A | 27-09-1990 |
| | | | CA 2012777 A1 | 23-09-1990 |
| | | | DE 69031237 D1 | 18-09-1997 |
| | | | DE 69031237 T2 | 02-01-1998 |
| | | | DE 389063 T1 | 10-10-1996 |
| | | | DK 389063 T3 | 30-03-1998 |
| | | | EP 0389063 A2 | 26-09-1990 |
| | | | EP 0819696 A2 | 21-01-1998 |
| | | | ES 2085245 T1 | 01-06-1996 |
| | | | GR 96300019 T1 | 31-03-1996 |
| | | | GR 3025351 T3 | 27-02-1998 |
| | | | JP 2289596 A | 29-11-1990 |
| | | | JP 2680462 B2 | 19-11-1997 |
| | | | JP 10072485 A | 17-03-1998 |
| | | | JP 2001078790 A | 27-03-2001 |
| | | | KR 148693 B1 | 01-08-1998 |
| | | | ZA 9002190 A | 24-12-1991 |
| ----- | | | | |
| US 6027945 | A | 22-02-2000 | AU 732756 B2 | 26-04-2001 |
| | | | AU 6647598 A | 07-08-1998 |
| | | | BR 9805897 A | 24-08-1999 |
| | | | CA 2249393 A1 | 23-07-1998 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 03/01202

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| US 6027945 | A | DE 895546 T1 | 17-10-2002 |
| | | EP 0895546 A1 | 10-02-1999 |
| | | JP 3253638 B2 | 04-02-2002 |
| | | JP 11509742 T | 31-08-1999 |
| | | US 2002086326 A1 | 04-07-2002 |
| | | WO 9831840 A1 | 23-07-1998 |
| | | US 6368800 B1 | 09-04-2002 |
| ----- | | | |
| WO 8706621 | A | 05-11-1987 | AT 114334 T |
| | | | 15-12-1994 |
| | | | AU 613870 B2 |
| | | | 15-08-1991 |
| | | | AU 7432987 A |
| | | | 24-11-1987 |
| | | | CA 1301606 C |
| | | | 26-05-1992 |
| | | | DE 3750774 D1 |
| | | | 05-01-1995 |
| | | | DE 3750774 T2 |
| | | | 27-04-1995 |
| | | | EP 0305399 A1 |
| | | | 08-03-1989 |
| | | | JP 2552691 B2 |
| | | | 13-11-1996 |
| | | | JP 1502317 T |
| | | | 17-08-1989 |
| | | | US 5482834 A |
| | | | 09-01-1996 |
| | | | WO 8706621 A1 |
| | | | 05-11-1987 |
| ----- | | | |